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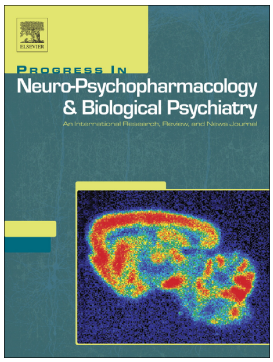

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Downregulation of plasma SELENBP1 protein in patients with recent-onset schizophrenia

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Abstract

Upregulation of selenium binding protein 1 (*SELENBP1*) mRNA expression has been reported in schizophrenia, primarily in the dorsolateral prefrontal cortex. However, peripheral blood studies are limited and results are inconsistent. In this study, we examined *SELENBP1* mRNA expression in whole blood and protein expression in plasma from patients with recent-onset schizophrenia (n=30), treatment-resistant schizophrenia (n=71) and healthy controls (n=57). We also examined the effects of *SELENBP1* genetic variation on gene and protein expression. We found lower SELENBP1 plasma protein level in patients with recent-onset schizophrenia (p=0.042) but not in treatment-resistant schizophrenia (p=0.81). Measurement of peripheral mRNA levels showed no difference between treatment-resistant schizophrenia and healthy controls (p=0.234) but clozapine plasma levels (p=0.036) and duration of illness (p=0.028) were positively correlated with mRNA levels. Genetic variation was not associated with mRNA or protein expression. Our data represent the first peripheral proteomic study of *SELENBP1* in schizophrenia and suggest that plasma SELENBP1 protein is downregulated in patients with recent-onset schizophrenia.

Keywords: SELENBP1, gene expression, protein expression, recent-onset schizophrenia, treatment-resistant schizophrenia, biomarker analysis.

1. Introduction

The selenium-binding protein 1 (*SELENBP1)* gene is located in chromosome 1q21.3 and encodes for a protein thought to be involved in mediating the effects of selenium in peripheral and central tissues (Bansal et al. , 1989, Glatt et al. , 2005, Yao et al. , 2008). *SELENBP1* has also been implicated in ubiquitin-mediated protein degradation in a selenium-dependent manner (Jeong et al. , 2009). Post-mortem human brain studies have reported upregulation of *SELENBP1* mRNA in the dorsolateral prefrontal cortex (DLPFC) of individuals with schizophrenia (Glatt, Everall, 2005, Kanazawa et al. , 2008, Udawela et al. , 2015). Similar evidence of upregulation of *SELENBP1* mRNA in peripheral blood is also available in individuals with chronic schizophrenia (Glatt, Everall, 2005) and two single nucleotide polymorphisms in the *SELENBP1* gene (rs1078804 and rs2769264) have been identified as susceptible loci for schizophrenia in a family-wide association study (Kanazawa et al. , 2009). Although, findings in schizophrenia have been mixed as negative results are also available. Among those with recent onset schizophrenia, no difference in peripheral mRNA expression was found relative to healthy controls (Yao, Schroder, 2008) and *SELENBP1* protein levels in the DLPFC of individuals with schizophrenia also did not differ compared to controls (Udawela, Money, 2015). However, the overall evidence available to make firm conclusions about the association between *SELENBP1* and schizophrenia, particularly specific sub-groups of schizophrenia is limited. Moreover, the effect of genetic mutations, lifestyle (smoking, alcohol use, substance abuse), and/or symptom severity on *SELENBP1* mRNA and protein expression is yet to be investigated.

The primary aim of this study was to measure peripheral *SELENBP1* mRNA and protein levels in individuals with treatment-resistant schizophrenia and those with recent onset schizophrenia and compare them to healthy controls. Aligned with our previous findings in post-mortem brain and peripheral blood of individuals with schizophrenia (Glatt, Everall, 2005), we hypothesized that

SELENBP1 mRNA and protein levels would be upregulated in schizophrenia relative to controls. Secondary aims of the study included exploration of whether expression levels are affected by variables such as age of illness onset, duration of illness, symptom severity, clozapine plasma level, and *SELENBP1* genetic variation.

2. Materials and Methods

2.1 Participants

The Structured Clinical Interview for DSM-IV Axis I Disorders (First et al. , 1997) or Mini International Neuropsychiatric Interview (MINI) (Sheehan et al. , 1998) were used to confirm diagnosis. Seventy-one schizophrenia patients were recruited from inpatient and outpatient clinics around Melbourne, Australia. All participants were aged between 18-65 years and were currently prescribed and taking clozapine. Per the current criteria, they were considered as 'treatment-resistant' as they failed to response to two or more previous trials of antipsychotics with persistent symptoms and poor functioning (Howes et al. , 2017). Thirty recent-onset schizophrenia patients (mean duration of illness < 1.5 years) were also recruited from clinical services in Melbourne as part of an ongoing neuroimaging study. A comparison group of fifty-seven healthy, unrelated controls was recruited from the general community and matched for age, gender and socio-economic status. Exclusion criteria for healthy controls included having a first-degree relative with psychiatric illness, neurological disease, current pregnancy/breast feeding, diabetes, head injury, seizures, prior or current use of antipsychotic medications, daily use of steroidal or nonsteroidal anti-inflammatory, immunosuppressive, corticoid or glucocorticoid drugs continuously for more than one week within the last month, generalized inflammatory condition/disease, premorbid IQ less than 70 with a documented history of developmental delay or intellectual disability, thyroid dysfunction and substance abuse or dependence. In addition, recent onset

individuals were excluded if psychotic symptoms were entirely explained by drug use. Table 1 contains the details demographics of the participants.

After an overnight fast, whole blood was collected and processed following the standard blood collection and processing protocol (see supplementary methods for more details). Participants were provided with written information and written consent was sought form all eligible individuals prior to participation. This study was approved by the Melbourne Health Human Research Ethics Committee (MHREC ID 2012.069 and 2012.066) and complied with the Declaration of Helsinki and its subsequent revisions (World Medical Association., 2013).

2.2 Clinical measures

To assess clinical symptoms, the Positive and Negative Syndrome Scale (PANSS) (Kay et al. , 1987) was used and the patients were scored according to the consensus five factor (positive, negative, disorganised, excitement, depression) PANSS model (Wallwork et al. , 2012) in the treatment-resistant schizophrenia (TRS) cohort. Data on Illicit drug use, tobacco, and alcohol consumption in the past three months was collected using a standard substance use questionnaire. In the recent onset schizophrenia patients, the Expanded Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) was used to assess positive symptom severity. BPRS scores were converted to PANSS scores as previously described (Leucht et al. , 2013) to allow for consistent analysis of symptoms across cohorts. Current/last chlorpromazine equivalent dosage was calculated in all patients by following standard guidelines (American Psychiatric Association., 1997, Woods, 2003) and among the treatment-resistant schizophrenia participants, clozapine plasma levels were measured.

2.3 Selection of SNPs, DNA extraction and genotyping

Five haplotype tagging-single nucleotide polymorphisms (SNPs) which spanned the *SELENBP1* gene were selected (rs10788804, rs2769264, rs2800953, rs2864118 and rs1752380) using the Northern and Western European ancestry (CEU) population as reference from the International Haplotype Map (HapMap) Project along with 60 unlinked ancestry informative markers representing the three HapMap phase III populations (Northern/Western European, Han Chinese, Yoruba in Nigeria) (Enoch et al. , 2006). Supplementary Table S1 lists the SNPs and call rates. Two of these (rs1078804 and rs2769264) had been previously identified as being linked with schizophrenia (Kanazawa, Glatt, 2009). Genotyping was performed at the Australian Genome Research Facility (Brisbane, Australia) with the Sequenom MassARRAY MALDI-TOF genotyping system using Sequenom iPLEX Gold chemistries according to the manufacturer's instructions (Sequenom, Inc., San Diego, CA).

2.4 RNA extraction, cDNA synthesis and quantitative real-time PCR

In the TRS cohort, total RNA extraction and quantification from whole blood was conducted using PureLink® RNA mini kit (ThermoFisher Scientific, Waltham, MA, USA) following standard manufacturer's protocol. Total RNA was reverse transcribed to complementary DNA (cDNA) using SuperScript® IV First-Strand Synthesis System (Invitrogen, Foster city, CA, USA) using random hexamers. Complementary DNA (10.25ng) was used as a template for real-time PCR using master mix and gene specific validated Taqman assays from Applied Biosystems, Foster City, California, USA. Inventoried assays were used for *SELENBP1* as well as the four reference genes (beta-actin, *ACTB*; ubiquitin C, *UBC*; glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*; and TATA box binding protein, *TBP*). See Supplementary Table S2 for a list of each of the probes and primers.

For gene expression measurement, 10.25 ng of cDNA was subjected to real-time PCR in duplicate using FAM-MGB TaqMan® gene expression probes (Invitrogen, Foster city, CA, USA) in 192x24 Dynamic Arrays IFC in Fluidigm® BioMark™ HD system (South San Francisco, CA, USA) at the Monash Health Translation Precinct Medical Genomics Facility (Hudson Institute of Medical Research, Clayton, VIC, Australia). In addition, no reverse transcriptase controls and no template controls were included to rule out genomic DNA contamination and reagent contamination, respectively. Adhering to minimum information for publication of RT-qPCR (MIQE) guidelines (Bustin et al. , 2009), normalized relative quantities (NRQ, i.e. $2^{-\Delta Ct}$ where $\Delta C_t = Ct_{(candidate\ gene)} - Ct_{(geometric\ mean\ of\ reference\ genes)}$) of *SELENBP1* mRNA was calculated using the geometric mean expression of two reference genes (*ACTB and UBC*) that did not differ between groups. *GAPDH* and *TBP* were not used as reference genes because their expression differed significantly by group in the TRS cohort (Supplementary Figure S1).

2.5 Protein quantification

Human SELENBP1 ELISA kits (Catalogue number: abx153044) were obtained from Abbexa Ltd. (Cambridge, UK) and protein quantification in plasma was performed following the manufacturer's protocol. Briefly, 100 µL of diluted plasma (1:4 dilution with 0.01mol/L PBS; p^H=7.0-7.2) from both cohorts and standards (0.156 ng/mL-10 ng/mL) were added to the wells in duplicate. The ELISA kits have a sensitivity of <0.054 ng/mL. Absorbance was measured on a SpectraMax® M3 multi-mode microplate reader (Molecular Devices, LLC; Sunnyvale, CA, USA) at 450nm wavelength. The standard curve ($r^2 \geq 0.99$) was generated by plotting relative absorbance of each standard solution on the Y-axis vs the respective concentration of the standard solution on the X-axis using a four-parameter logistic

curve fit. The concentration of SELENBP1 protein in plasma samples was obtained by interpolating the absorbance values using the standard curve in GraphPad Prism 7. The final protein values were calculated after multiplying with the dilution factor (DF=4).

2.6 Statistical Analysis

Two-tailed tests were used for all statistical analyses. Quantile-quantile (Q-Q) plots and Shapiro-Wilk test were used to examine the normality of variable distributions. Independent sample t-tests were used to test differences for continuous variables between groups, while chi-squared tests (χ^2) were used for categorical variables. To adjust for multiple comparisons, Benjamini-Hochberg step-up procedure (Benjamini and Hochberg, 1995) was used and effect size was calculated using the Hedges' g method (Hedges and Olkin, 1985).

2.7 SELENBP1 mRNA and protein analysis

The normalized relative quantity for *SELENBP1* mRNA transcript as well as SELENBP1 protein values were checked for normality using Q-Q plots (Supplementary Figure S2). In addition, we assessed the following variables as potential confounders: age, gender, RNA integrity number (for mRNA only), alcohol use, and smoking status. A variable was considered a confounder and included in our statistical models only when it was significantly different between groups ($p<0.05$) and was significantly associated with gene or protein expression. The normalised mRNA values were compared among groups (TRS cohort only) using generalized linear models based on their distribution and adjusted for appropriate covariates. Protein values were compared between all three groups (TRS, recent onset schizophrenia, and control) using the Kruskal-Wallis test. The difference between groups were measured by *post hoc* pairwise comparison. Outliers were identified using the Grubbs' test for outliers and removed from further analysis.

Spearman correlations were calculated between gene transcript/protein levels and symptom severity, age of illness onset, illness duration, current chlorpromazine equivalent dose, and clozapine plasma levels. In the TRS cohort, mRNA transcript/protein levels between participants in positive symptom remission and non-remission were compared using a Mann-Whitney U test. Positive symptom remission was defined as a PANSS score of ≤3 on delusions, hallucinations, grandiosity and unusual thought content (Wallwork, Fortgang, 2012).

2.8 SNP/haplotype analysis

The GRCh38/hg19 human genome reference assembly was used to map the *SELENBP1* SNPs. Linkage disequilibrium (LD) between SNPs was examined in Haploview (Supplementary Figure S3) and haplotype blocks determined using the solid spine method (Barrett et al. , 2005). For each individual, haplotypes were determined based on the best posterior probability procedure implemented in PLINK 1.07 (Purcell et al. , 2007). Generalised linear models were used to explore cis-regulatory effects of each SNP and haplotype on mRNA transcript and protein expression. Each model included genotype/haplotype, case status, genotype/haplotype x case status as well as other relevant covariates (age, gender, RIN, smoking, alcohol). Significant genotype/haplotype x case status interactions were analysed *post hoc* by case status stratification analyses.

3. Results

3.1 SELENBP1 mRNA and protein expression

There was no difference for *SELENBP1* mRNA level between individuals with treatment-resistant schizophrenia and healthy controls (p_{raw}= 0.234). However, Kruskal-Wallis test of SELENBP1 protein showed variance with diagnosis (H=11.756, df=2, p=0.003).

There was 15% lower level of plasma SELENBP1 protein in those with recent onset schizophrenia compared to healthy controls (p_{raw} = 0.014; p_{adj} = 0.042) (Figure 1). We found no difference for plasma SELENBP1 protein level between TRS individuals and controls (p_{raw}=0.81) or between the TRS cohort and recent onset schizophrenia patients (p_{raw} = 0.171).

3.2 SELENBP1 mRNA/protein expression and symptomatology

We found a statistically significant positive correlation of plasma SELENBP1 protein level with duration of illness (Spearman's rho = 0.292, p_{adj} = 0.028) and clozapine plasma level (Spearman's rho = 0.253, p_{adj} = 0.036) in the treatment-resistant schizophrenia patients (Figure 2; Supplementary Table S3). There was no significant correlation for mRNA or protein level with symptom severity (Supplementary Table S4). Our exploratory analysis showed no difference for *SELENBP1* mRNA and protein expression between individuals who had positive symptoms compared to those with non-remission (p=0.754 and 0.610 respectively).

3.3 Genotype/haplotype effect on mRNA and protein expression

We found trend level association for SNP rs2864118 (p_{raw} = 0.013; $P_{B\text{-}H}$ = 0.117) and haplotype block TCT (p_{raw} = 0.013; p_{adj} = 0.117) with SELENBP1 protein expression in plasma in TRS patients. However, none of these associations survived correction for multiple comparisons (Supplementary Table S5).

4. Discussion

In the present study, we found no difference in peripheral *SELENBP1* mRNA expression between individuals with treatment-resistant schizophrenia and healthy controls. However, we observed that plasma SELENBP1 protein level was lower in those individuals with

recent-onset schizophrenia. In the TRS individuals, SELENBP1 protein expression was confounded by clozapine plasma level and was positively correlated with duration of illness. Furthermore, we found none of the SNPs or haplotypes acted as expression quantitative trait loci (eQTL) for mRNA or protein expression.

We did not find statistically significant difference for peripheral *SELENBP1* mRNA expression between TRS patients and healthy controls. This findings does not support our hypothesis but is in agreement with a previous study that found similar outcome for this transcript in peripheral blood mononuclear cells in individuals experiencing their first hospitalisation with a schizophrenia spectrum disorder (schizophrenia, schizoaffective or schizophreniform disorder) (Yao, Schroder, 2008). Collectively, this suggests that peripheral mRNA expression pattern of *SELENBP1* may not be dependent on disorder state. Both studies investigated populations predominantly of Caucasian ancestry. Although one study reported an increased peripheral *SELENBP1* mRNA expression in Han Chinese individuals with schizophrenia (Glatt, Everall, 2005), suggesting that ethnicity may play an important role for differential expression pattern of this transcript in schizophrenia. Several post-mortem human brain studies have demonstrated upregulation of *SELENBP1* mRNA in the DLPFC in schizophrenia (Glatt, Everall, 2005, Kanazawa, Chana, 2008, Udawela, Money, 2015). Overall, this leads to the idea that there is tissue specific differential expression pattern for *SELENBP1* mRNA in schizophrenia. Thus, the utility of using *SELENBP1* mRNA as a peripheral biomarker in clinical settings needs to be validated in larger cohorts with populations from different ethnic backgrounds and disorder state.

The novel finding of our study was of a lower level of plasma SELENBP1 protein in individuals with recent-onset schizophrenia. Although this findings does not support our *a priori* hypothesis, it is in line with previous studies that have shown selenium (Se) deficiency was associated with higher rates of schizophrenia (Jnr, 1994, Joyce, 1987) and lower plasma Se concentrations in patients with

schizophrenia (Arinola and Idonije, 2009, Vaddadi et al. , 2003). SELENBP1 binds selenium (Bansal et al. , 1990, Chang et al. , 1997), a trace element involved in neuroprotection (Dalla Puppa et al. , 2007, Porciuncula et al. , 2001, Yeo and Kang, 2007) but the primary function of SELENBP1 is yet to be identified as it is not a member of the selenoprotein family. The role of selenium and selenoproteins in brain are diverse, ranging from antioxidant and anti-inflammatory effects to phosphorylation of proteins and ion channels as well as brain cholesterol metabolism (Rayman, 2012, Solovyev, 2015). Two trace element studies showed no difference in plasma Se concentration between patients with schizophrenia and controls but SELENBP1 protein levels were not measured (Vidovic et al. , 2013, Yanik et al. , 2004). SELENBP1 protein expression was also found to be lower in post-mortem liver from individuals with schizophrenia (Prabakaran et al. , 2007), thereby indicating that, lower peripheral protein expression of SELENBP1 may be a unique signature in schizophrenia. Moreover, we found that SELENBP1 protein expression was trend-level lower in those with treatment-resistant schizophrenia relative to healthy controls but is slightly higher in TRS compared to recent-onset schizophrenia, although neither was statistically significant. These findings support the dynamic nature of schizophrenia and suggest that SELENBP1 protein expression is dependent on disorder state by which transition into full blown symptoms may lead to reduced protein expression in plasma that later in the illness returns to or in some cases exceeds control levels. Longitudinal studies will be required to test this notion.

Our finding of no difference in peripheral SELENBP1 protein expression between TRS and healthy controls supports a previous post-mortem human brain study that reported no difference for protein expression in the DLPFC in schizophrenia. A caveat of that study was that they did not include post-mortem human brain samples from recent-onset schizophrenia patients (Udawela, Money, 2015) and so we cannot confirm whether the same trajectory of lower SELENBP1 protein expression will be a characteristic feature in brain tissue in

early stage of schizophrenia. To the best of our knowledge, we are the first group to report decreased plasma SELENBP1 protein level in recent-onset schizophrenia and so it needs to be replicated and validated in other independent cohorts with larger sample size.

Interestingly, our analyses showed that plasma SELENBP1 protein expression was positively correlated with clozapine plasma level in treatment-resistant patients. Clozapine is the medication of choice in TRS and may be responsible for causing upregulation of SELENBP1 protein in plasma. This hypothesis is supported by our finding of trend-level higher protein expression in TRS patients compared to recent-onset schizophrenia patients as none of the recent-onset schizophrenia patients were on clozapine. A previous study by Udawela et al. reported that animal models treated with chlorpromazine, thioridazine, and haloperidol had no effect on *SELENBP1* mRNA level in the CNS (Udawela, Money, 2015). Future studies should examine as to how clozapine causes changes in mRNA and protein expression in the CNS and periphery to clarify if changes in transcript and/or protein level are due to schizophrenia or is a drug effect. Furthermore, we found positive correlation of SELENBP1 protein expression with duration of illness in TRS patients, indicating that as the illness progresses the upregulation of protein becomes more evident. However, it is not clear whether this correlation represents a consequence of disease progression and/or a compensating effect to maintain normal cellular functions. Studies examining the pattern of SELENBP1 protein expression throughout the course of illness are required to verify the notion and discover the underlying mechanism.

Our study had several limitations. First, we did not have mRNA measurements for *SELENBP1* in recent-onset schizophrenia individuals and thus cannot confirm if lower SELENBP1 protein level was consequence of lower mRNA levels in recent-onset schizophrenia. Second, we did not have matched controls for recent-onset schizophrenia patients and we utilised a control group with a higher mean age group. Although, we adjusted for age in all our analysis we cannot completely rule out the possibility that older controls may have resulted in potential bias in the results. Third, we used whole blood for mRNA measurements and plasma for protein

measurement in the TRS cohort. It is not clear how our findings will relate to other accessible peripheral tissues (i.e. serum, lymphocytes, peripheral blood cells) commonly used in biomarker research. Furthermore, we were unable to determine what relationship, if any, peripheral levels of *SELENBP1* have with brain levels as we did not have access to brain tissue from individuals with recent-onset schizophrenia. Fourth, our sample size was relatively small and our results should to be replicated and validated in independent cohorts. Fifth, we used populations predominantly of Caucasian ancestry and thus future investigation with population from other ethnic backgrounds are warranted.

In summary, our results provide the first protein expression profile of plasma SELENBP1 in recent-onset schizophrenia as well as treatment-resistant schizophrenia. We observed a downregulation of plasma SELENBP1 protein among those with recent-onset schizophrenia. We further showed that increase in SELENBP1 protein expression in TRS may be regulated by clozapine plasma level as well as duration of illness suggesting that drug exposure and illness duration may have profound effect on protein expression. Further studies are required to verify if *SELENBP1* can be used as a candidate biomarker for symptom remission and symptom severity in schizophrenia.

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4.2 Conflicts of interest

The authors declare no conflicts of interest.

4.3 Highlights

- Plasma SELENBP1 protein level was decreased in patients with recent-onset schizophrenia.

- No difference in peripheral *SELENBP1* mRNA expression was detected between patients with treatment-resistant schizophrenia and healthy controls.
- Peripheral *SELENBP1* mRNA expression was positively correlated with clozapine plasma level and duration of illness in patients with treatment-resistant schizophrenia.

Ethical Statement

All participants in this study were provided with written information and written consent was sought form all eligible individuals prior to participation. This study was approved by the Melbourne Health Human Research Ethics Committee (MHREC ID 2012.069 and 2012.066) and complied with the Declaration of Helsinki and its subsequent revisions.

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Figure 1: *SELENBP1* mRNA and protein expression: **(a)** normalised relative quantities (NRQ) of *SELENBP1* mRNA [treatment-resistant schizophrenia (TRS): 0.11, interquartile range (IQR)= 0.06-0.19; controls: 0.12, IQR: 0.06-0.21; Wald chi-square (χ^2) =1.416, df=1, p_{raw}=0.234, Hedges' g= 0.30)]; **(b)** plasma protein level of SELENBP1 [TRS: 1.84 ng/mL, IQR=1.33-2.41; recent onset schizophrenia: 1.74 ng/mL, IQR=1.01-2.10; controls: 2.03 ng/mL, IQR=1.69-2.70; TRS vs recent-onset schizophrenia: Wald chi-square (χ^2) =1.21, df=1, p_{raw} = 0.171, g=0.51 ; TRS vs controls: Wald chi-square (χ^2) =2.77, df=1, p=0.81, g=0.30; recent-onset schizophrenia vs controls: Wald chi-square (χ^2) =6.16, df=1, p_{raw}=0.014, p_{adj}=0.042, g=0.90). Error bars represent median ± interquartile range. * p<0.05.

Figure 2: The correlation of SELENBP1 protein with clinical factors in treatment-resistant schizophrenia patients: **(a)** correlation of SELENBP1 protein with clozapine plasma levels (Spearman's rho = 0.253, p_{adj}= 0.036), **(b)** correlation of SELENBP1 protein with duration of illness (Spearman's rho=0.292, p_{adj}= 0.028).

Table 1: Demographic data and clinical characteristics of participants

Characteristic	1. Treatment-resistant schizophrenia (n=71)	2. Recent onset schizophrenia (n=30)	3. Controls (n=57)	1 vs 2	1 vs 3	2 vs 3
Age, mean (sd) years	40 (10)	21 (2)	40 (11)	**<0.001**[a]	0.702[a]	**<0.001**[a]
Gender, n (%) males	53 (75)	23 (77)	35 (61)	1.000[b]	0.108[b]	0.231[b]
RNA integrity number, mean (sd)	8.4 (0.9)	-	8.7 (0.3)	-	**0.006**[a]	-
Ancestry, n (%) CEU	62 (90)	23 (77)	50 (88)	0.189[b]	0.742[b]	0.165[b]
Substance use in past three months, n (%)						
Tobacco (smoked)	33 (47)	18 (60)	12 (21)	0.157[b]	**0.003**[b]	**<0.001**[b]
Alcohol	59 (83)	-	55 (97)	-	**0.016**[b]	-
Cannabis	11 (15)	-	7 (12)	-	0.385[b]	-
Amphetamine	4 (6)	-	2 (4)	-	0.439[b]	-
Cocaine	0 (0)	-	2 (4)	-	0.137[b]	-
Opiates	1 (1)	-	1 (2)	-	0.990[b]	-
Clozapine plasma level, mean (sd) µg/L	432 (234)	-	-		-	
Chlorpromazine equivalent (excluding clozapine) dosage mean (sd) mg/day	142 (286)	338 (312)	-	-	-	-
Age of onset, mean (sd) years	22.5 (6)	19.5 (2)	-		-	-
Duration of illness, mean (sd) years	17 (8)	1.12 (1)	-		-	-
PANSS scores, mean (sd)						-
Positive	10 (6)	-	-		-	-
Negative	15 (5)	-	-		-	-
Disorganized	8 (3)	-	-		-	-
Excitement	6 (2)	-	-		-	-
Depression	6 (3)	-	-		-	-
Total	62 (14)	58 (16)	-		-	-

RIN=RNA integrity number; PANSS=Positive and Negative Syndrome Scale; mg=milligram.
[a]Independent samples t-test , [b] Chi-square(χ^2) test, $p<0.05$ values are made bold

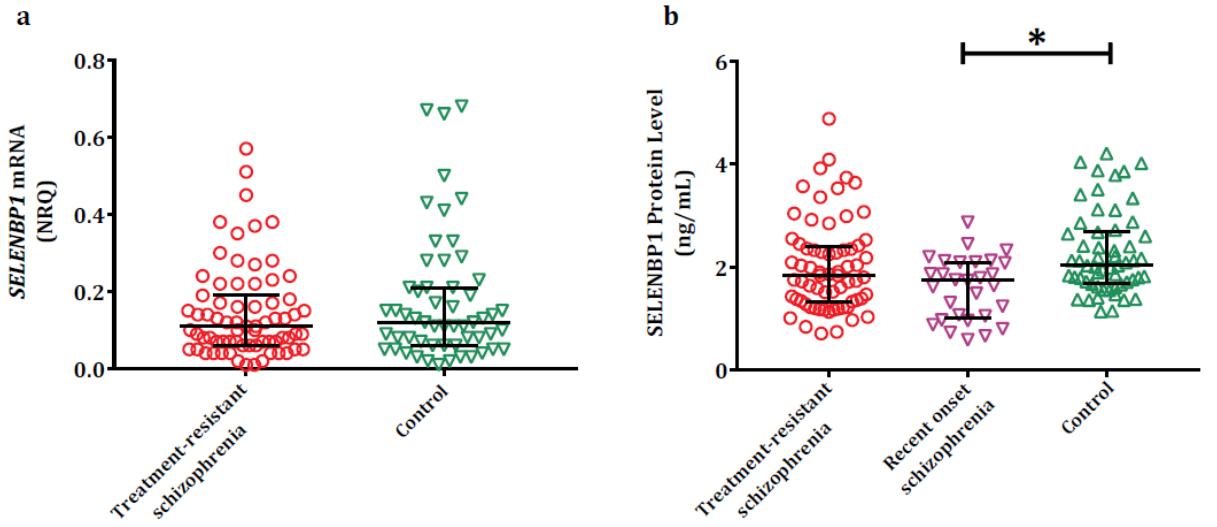

Fig: 1

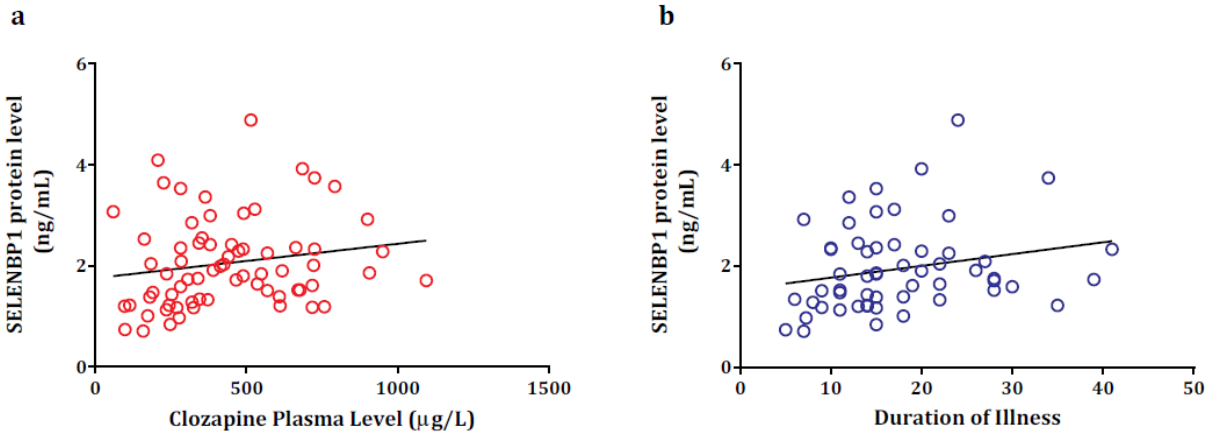

Fig: 2